

HERV-W Envelope Triggers Abnormal Dopaminergic Neuron Process through DRD2/PP2A/AKT1/GSK3 for Schizophrenia Risk

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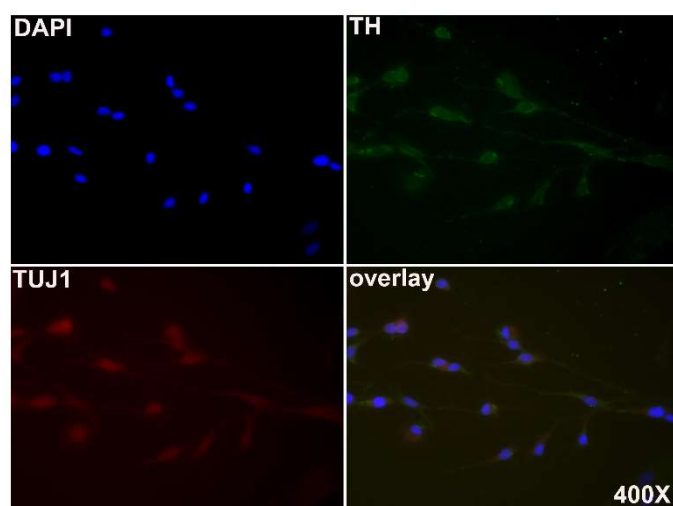
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Supplementary Figure S1. Immunofluorescence staining of rat primary dopaminergic neurons. The primary dopaminergic neurons were isolated from the rat midbrain VTA (in the middle of midbrain) and cultured in vitro. The primary neurons were identified by immunofluorescence with the double-staining with TH and TUJ1 antibodies. Magnification, 400×.

Supplementary Table S1. Human primer sequences.

Name	Sequence	Length (bp)
<i>HERV-W ENV-F</i>	CCATGCCGCTGTATGACCAG	109
<i>HERV-W ENV-R</i>	GGGTCCCTTAGAAAGACTCCT	
<i>SNAP23-F</i>	TGGAAGAGAACCTGACTCAAGT	120
<i>SNAP23-R</i>	CAGCCTTGTCTGTGATTCGTTT	
<i>SNAP25-F</i>	TCGTGTAGTGGACGAACGG	158
<i>SNAP25-R</i>	TCTCATTGCCCATATCCAGGG	
<i>SYN1-F</i>	AGCTCAACAAATCCCAGTCTCT	103
<i>SYN1-R</i>	CGGATGGTCTCAGCTTTCAC	
<i>SNCA-F</i>	TGTAGGCTCCAAAACCAAGG	139
<i>SNCA-R</i>	TGCTCCCTCCACTGTCTTCT	
<i>GAP43-F</i>	GGCCGCAACCAAAATTCAGG	167
<i>GAP43-R</i>	CGGCAGTAGTGGTGCCTTC	
<i>TH-F</i>	CGACCCTGACCTGGACTTGGA	142
<i>TH-R</i>	GGCAATCTCCTCGGCGGTGT	
<i>DAT-F</i>	CCATACTGAAAGGTGTGGGC	146
<i>DAT-R</i>	CCAGGAGTTGTTGCAGTGGA	
<i>SYP-F</i>	GAAGGTGCTGCAATGGGTCT	143
<i>SYP-R</i>	GCCTGAAGGGGTACTCGAAC	
<i>VAMP1-F</i>	ACATGACCAGTAACAGACGACT	76
<i>VAMP1-R</i>	ACGTTACACGTATGATGTCC	
<i>VMAT2-F</i>	TGGACAACATGCTGCTCACTG	137
<i>VMAT2-R</i>	GCTCTGGAAGCTGTCTGAGATGG	
<i>PSD93-F</i>	GCCGGTGATTATCCTGGGG	108

<i>PSD93-R</i>	CGCTTTGGCCTCGTAGTATGA	120
<i>PSD95-F</i>	CTCGATGTCTCGGCCAATG	
<i>PSD95-R</i>	CTCTGTGATCCGCTTGTTAATCTC	
<i>DRD2-F</i>	CCCCGCCAAACCAGAGAAG	89
<i>DRD2-R</i>	TTTTGCCATTGGGCATGGTCT	
<i>GAPDH-F</i>	CTGGGCTACACTGAGCACC	101
<i>GAPDH-R</i>	AAGTGGTCGTTGAGGGCAATG	

Supplementary Table S2. Rat primer sequences.

Primer	Sequence	Length (bp)
<i>SNAP23-F</i>	GGGGAACAACATAATCG	183
<i>SNAP23-R</i>	GCTGTCTCCACCATCAC	
<i>SNAP25-F</i>	TGGCTGATGAGTCCCT	109
<i>SNAP25-R</i>	AGTTGTTGCGCCTTGCT	
<i>SYN1-F</i>	CCTCGCTGTCTAACGC	164
<i>SYN1-R</i>	CCCTTTGAAGTATTTTGC	
<i>SNCA-F</i>	CGTCCTCTATGTAGGTTC	85
<i>SNCA-R</i>	TGTCATTGTTCTTTGGTC	
<i>GAP43-F</i>	AGAAGGGCGAAGGGGATG	100
<i>GAP43-R</i>	TTGGAGGACGGCGAGTT	
<i>TH-F</i>	CCTTCCAGTACAAGCACGGT	109
<i>TH-R</i>	TGGGTAGCATAGAGGCCCTT	
<i>DAT-F</i>	CCTATGGAAGGGAGTAAA	159
<i>DAT-R</i>	TCACAGAGTCGGTAGAAGT	
<i>SYP-F</i>	GTGCCAACAAGACGGAG	99
<i>SYP-R</i>	CTTTGACGCAGGAGGGT	
<i>VAMP1-F</i>	AGACGATTACAGCAAACC	106
<i>VAMP1-R</i>	CATCCAACCTCTGACAACTT	
<i>VMAT2-F</i>	CTCCTGCTCATCCGTG	208
<i>VMAT2-R</i>	AATAGCCCCATCCAAG	
<i>PSD93-F</i>	GCCAAGAAAACCTATGACCG	155
<i>PSD93-R</i>	TTGAGGGAATCCAGATGAAA	
<i>PSD95-F</i>	CAACACGGACACCCTA	102
<i>PSD95-R</i>	CCTGAGTTACCCCTTTC	
<i>DRD2-F</i>	TGGTGGGTGAGTGGAA	108
<i>DRD2-R</i>	TGTCAATGCTGATGGC	
<i>GAPDH-F</i>	GCATCTTCTTGTGCAGTGCC	105
<i>GAPDH-R</i>	GAGAAGGCAGCCCTGGTAAC	

Supplementary Table S3. Antibodies and the dilutions.

Proteins	Brands	Catalogs	Dilutions
TH	Abcam	ab137869	1:5000 (WB), 1:100 (IF)
TUJ1	Abcam	ab78078	1:150 (IF)

GAPDH	Abcam	ab8245	1:10000 (WB)
HERV-W ENV	Abcam	ab179693	1:1000 (WB), 1:100(IP)
DRD2	Abcam	ab85367	1:1000 (WB), 1:200 (IP)
GAP43	Abcam	ab75810	1:100000 (WB)
DAT	Abcam	ab111468	1:1000 (WB)
SYP	Abcam	ab14692	1:1000 (WB), 1:100 (IF)
VAMP1	Abcam	ab151712	1:5000 (WB)
VMAT2	Abcam	ab70808	1:1000 (WB)
PSD93	Abcam	ab94588	1:1000 (WB)
PSD95	Abcam	ab18258	1:1000 (WB), 1:200 (IF)
ARRB2	Abcam	ab206972	1:1000 (WB)
PP2A	Abcam	ab32104	1:5000 (WB)
AKT1	Abcam	ab227100	1:2000 (WB)
AKT1 p-S473	Abcam	ab81283	1:5000 (WB)
AKT1 p- T308	Abcam	ab105731	1:2000 (WB)
GSK3A	Abcam	ab40870	1:5000 (WB)
GSK3A p- S21	Abcam	ab131112	1:1000 (WB)
GSK3B	Abcam	ab131356	1:1000 (WB)
GSK3B p- S9	Abcam	ab131097	1:1000 (WB)

Supplementary Table S4. Gene ontology analysis of differentially expressed genes in schizophrenia.

Category	Term	Count	%	p-Value	FDR
GOTERM_BP_DIRECT	inflammatory response	21	0.0	3.1E-3	9.7E-1
GOTERM_BP_DIRECT	protein phosphorylation	22	0.0	1.1E-2	9.6E-1
GOTERM_BP_DIRECT	cell adhesion	22	0.0	1.2E-2	9.4E-1
GOTERM_BP_DIRECT	immune response	20	0.0	1.9E-2	9.7E-1
GOTERM_BP_DIRECT	negative regulation of cell proliferation	18	0.0	3.8E-2	9.8E-1
GOTERM_BP_DIRECT	sodium ion transmembrane transport*	6	0.0	4.6E-2	9.9E-1
GOTERM_CC_DIRECT	Protein complex	22	0.0	3.3E-3	7.1E-1
GOTERM_CC_DIRECT	Cell surface	26	0.0	5.3E-3	6.3E-1
GOTERM_CC_DIRECT	integral component of plasma membrane	52	0.1	1.5E-2	7.5E-1
GOTERM_MF_DIRECT	Mitogen-activated protein kinase binding	5	0.0	9.5E-4	5.0E-1
GOTERM_MF_DIRECT	Protein serine/threonine kinase activity	21	0.0	3.2E-3	6.8E-1
GOTERM_MF_DIRECT	Protein kinase activity	20	0.0	4.2E-3	5.4E-1

GOTERM_MF_DIRECT	ATP binding	56	0.1	1.2E-2	8.1E-1
GOTERM_MF_DIRECT	Transcription regulatory region DNA binding	13	0.0	1.3E-2	8.0E-1
GOTERM_MF_DIRECT	Calcium ion binding	29	0.0	3.3E-2	8.9E-1

Note: *the terms labeled bold are vital points in this study.

Supplementary Table S5. KEGG pathway analysis of differentially expressed genes in schizophrenia.

Category	Term	Count	%	p-Value	FDR
KEGG_PATHWAY	Hippo signaling pathway*	11	0.0	9.4E-3	8.8E-1
KEGG_PATHWAY	TNF signaling pathway	9	0.0	9.8E-3	6.7E-1
KEGG_PATHWAY	Adipocytokine signaling pathway	6	0.0	4.5E-2	9.7E-1
KEGG_PATHWAY	Adherens junction	6	0.0	4.8E-2	9.4E-1

Note: *the term labeled bold is regulated by Protein kinase, protein phosphorylation and protein serine/threonine kinase.

Supplementary Table S6. Comparison of demographic and clinical characteristics between the normal /onset schizophrenia (SCZ) groups in Chinese.

Group	Gender (n)		Age ($\bar{x} \pm S$)	BMI ($\bar{x} \pm S$)	Education years (n)					Smoking (n)	
	-----				-----					-----	
	Male	Female			3	5	9	12	16	Yes	No
SCZ patients (57)	24	33	42.16±12.78	21.54±2.66	3	4	12	24	14	20	37
Healthy controls (68)	31	37	43.26±11.62	21.39±3.48	3	8	17	23	17	26	42
t/X ²	0.153		0.507	0.284	1.551					0.132	
P value	0.417		0.613	0.777	0.818					0.852	

Supplementary Table S7. The concentration of DA in control and SCZ.

Groups	Mean	Median	Range	Minimum	Maximum	Std. Deviation	Skewness	Std. Error Skewness
Control (n=68)	3990.7	3516.1	5607.8	1996.6	7604.5	1296.6	0.71	0.7
SCZ (n=57)	15606.3	14776.3	32155.6	3931.5	36087.1	7023.9	0.41	0.4

Supplementary Table S8. The concentration of DRD1 in control and SCZ.

Groups	Mean	Median	Range	Minimum	Maximum	Std. Deviation	Skewness	Std. Error Skewness
Control (n=68)	17.96	16.76	63.06	2.13	65.19	8.78	3.43	3.35
SCZ (n=57)	16.88	15.91	50.14	2.97	53.10	7.13	2.41	2.34

Supplementary Table S9. The concentration of DRD2 in control and SCZ.

Groups	Mean	Median	Range	Minimum	Maximum	Std. Deviation	Skewness	Std. Error Skewness
Control (n=68)	1294.2	1311.1	3307.7	122.2	3429.8	661.5	0.28	0.27
SCZ (n=57)	8825.1	3732.4	79572.7	429.2	80001.9	14137.2	3.4	3.3

Supplementary Table S10. The concentration of HERV-W ENV in control and SCZ.

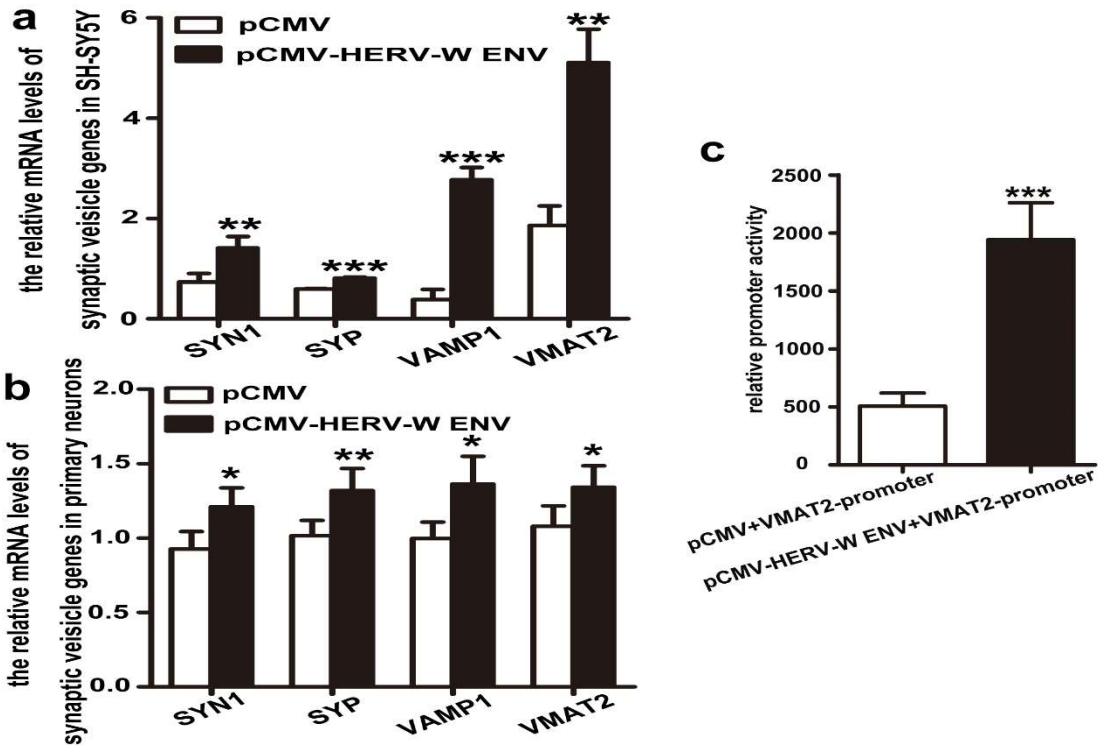
Groups	Mean	Median	Range	Minimum	Maximum	Std. Deviation	Skewness	Std. Error Skewness
Control (n=68)	4538.1	3950.6	26909	522.5	27431.5	3623.3	3.8	3.7
SCZ (n=57)	15246.8	8786.2	144777.9	1658	146435.9	24114.2	3.7	3.6

Supplementary Table S11. The concentration of dopamine in the HERV-W ENV (-) and HERV-W ENV (+) SCZ patients.

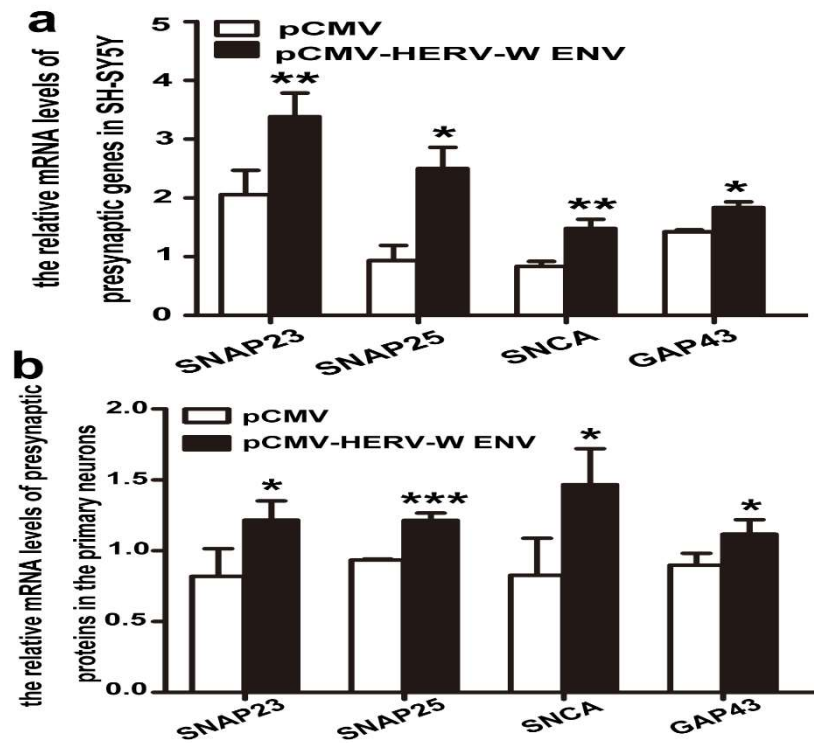
Groups	Mean	Median	Range	Minimum	Maximum	Std. Deviation	Skewness	Std. Error Skewness
HERV-W ENV (-)	12585.5	10389.2	21735.6	4376.1	26111.7	5950.1	0.8	0.8
HERV-W ENV (+)	17966.3	18654	32155.6	3931.5	36087.1	6893.5	0.1	0.1

Supplementary Table S12. The concentration of DRD2 in the HERV-W ENV (-) and HERV-W ENV (+) SCZ patients.

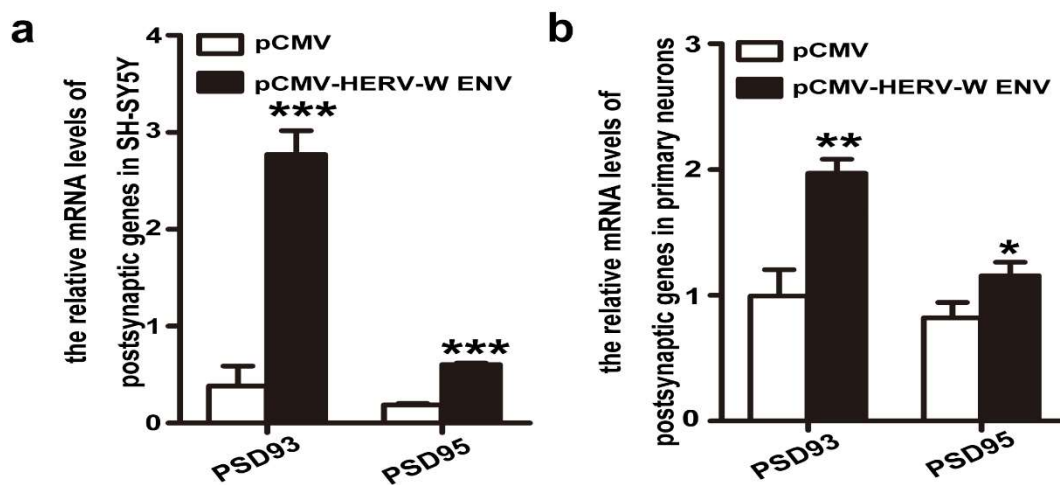
Groups	Mean	Median	Range	Minimum	Maximum	Std. Deviation	Skewness	Std. Error Skewness
HERV-W ENV (-)	1872.5	1944.6	2177.6	429.2	2606.7	447.7	1.2	1.1
HERV-W ENV (+)	14256.9	8710.4	77555.1	2446.8	80001.9	16987.5	2.6	2.5



Supplementary Figure S2. HERV-W ENV increases the mRNA levels of vesicle-related proteins and the promoter activity of VMAT2. (a) Real-time PCR for the synaptic vesicle-related proteins in SH-SY5Y cells. (b) Real-time PCR for the synaptic vesicle-related proteins in the primary dopaminergic neurons. (c) Luciferase assays of *pGL3-VMAT2* promoter co-transfected with *pCMV-HERV-W ENV* or control vector in SH-SY5Y cells.



Supplementary Figure S3. HERV-W ENV increases the mRNA levels of presynaptic proteins. (a) Real-time PCR for the presynaptic proteins in SH-SY5Y cells. (b) Real-time PCR for the presynaptic proteins in the primary dopaminergic neurons.



Supplementary Figure S4. HERV-W ENV increases the mRNA levels of postsynaptic proteins. (a) Real-time PCR for the postsynaptic proteins in the SH-SY5Y cells. (b) Real-time PCR for the postsynaptic proteins in the primary dopaminergic neurons.